RHODE ISLAND
DEPARTMENT OF HEALTH
LABORATORIES

MYCOBACTERIOLOGY LABORATORY

A Guide to Laboratory Services
for Mycobacteriology

Revised July 2004

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Governor

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Rhode Island HEALTH Laboratories
Mycobacteriology Laboratory
50 Orms Street
Providence, RI 02904-2284
Phone (401) 222-5587
Fax (401) 222-4572

This booklet was prepared as an aid in understanding the policies and procedures of the Mycobacteriology Laboratory.

Laboratory Business Hours: 8:30 a.m. – 4:30 p.m., Monday – Friday

The Laboratory observes Official State Government holidays.

Parking: Pick-up and delivery – Visitor parking available

Delivery of Specimens:

Fed Ex / UPS: Monday – Friday

State Courier Service: Monday – Friday

Delivery in Person: From 8:30 a.m. – 4:30 p.m. Monday – Friday
(Deliver to Receiving Room)

Specimen Transport Instruction

Specimens may be transported to Rhode Island HEALTH Laboratories (RIHL) via state or hospital courier services. The RIHL maintains courier service to each of the major community health centers and certain hospitals. Every clinical specimen submitted to the RIHL has the potential for transmitting disease. Specimens must be packaged to protect both the specimen and transporting personnel. Broken or leaking containers pose a safety hazard to transporting personnel and are unsatisfactory for testing.

Packaging requirements
Refer to following pages for complete packaging and transport instructions

Basic Materials:

- Use specimen container kits available from the RIHL whenever possible.
- Transport all specimens in double walled crush – proof packaging or in the special kits available for delivery by courier.
- Use appropriate slide mailing devices for transport of microscope slides.
- Use leak proof containers.
- Secure caps with waterproof tape or parafilm.
- Use polystyrene (or other insulating material) to ship frozen and refrigerated specimens.
- Use cold packs when transporting refrigerated specimens. Do not use wet ice.

Transport:

- The RIHL maintains a private courier service to the major providers.
- Hospitals may choose to use their own courier service.

Containers may be obtained by completing an order form from the following office:

Rhode Island HEALTH Laboratories
Receiving Room
50 Orms Street
Providence, RI 02904-2284
Phone: (401) 222-5548
Fax: (401) 222-6985

Test kit order form

MYCOBACTERIOLOGY

I. A. GENERAL INFORMATION

1. Specimen Collection:

    a. Optimally, all specimens should be collected prior to initiation of drug therapy.
    b. Specimens should be collected in containers provided by the RI HEALTH Laboratories. A sterile screw cap 50-ml centrifuge tube is appropriate for most specimens except blood and bone marrow.
    c. For sputum and other easily obtainable specimens, three to six single specimens on successive days are required.

2. Labeling of Specimens:

    The patient's name, laboratory requisition bar code label and date of collection **must** be affixed to each specimen. The specimen will not be tested if the specimen is unlabeled.

3. Packaging of Specimens:

    Primary specimens should be packaged for transport to the laboratory using the kits provided by the RI HEALTH Laboratories (RIHL). Please ensure that requisition forms are filled out completely with the information listed in section 4. **If an isolate is being submitted also include the primary smear result**. Place the specimen in one pouch of the plastic bag provided and the folded requisition form in the other side. Deliver to the laboratory as soon as possible.

4. Laboratory Requisition:

    There are several items of information that are necessary for **all** specimens. Specimen processing will be delayed until data can be obtained. The following information is needed:

    1. Submitter's name
    2. Referring Physician's license number and UPIN number
    3. Test requested, date of specimen collection
    4. Source (anatomical site) of the specimen
    5. Patient's name and address
    6. Date of birth, age, sex
    7. Insurance coverage
    8. Patient's signature on requisition form
    9. Diagnosis code

INSERT REQUISITION FORM HERE AND DISCARD THIS PAGE.

Patients should be instructed by attending medical personnel in methods and importance of proper specimen collection.

B. PULMONARY SPECIMENS

1. Sputum:
    a. Rinse mouth with water before sputum is collected to minimize food, mouthwash, and oral drugs, which may contaminate the specimen or inhibit growth of Mycobacterium
    b. Saliva and nasopharyngeal discharge is not appropriate for Mycobacterium testing
    c. Collect exudative material (sputum) brought up from lungs after deep productive cough
    d. Three consecutive day first morning specimens of 5-15 ml should be submitted for testing. **Do not pool specimens.**
    e. Sputum induction methods may be employed in patients who have great difficulty producing sputum. This is usually accomplished via the inhalation of warm, aerosolized, hypertonic (5-10%) saline. This causes sufficient irritation to the lungs to induce coughing and produce thin, watery specimens. It is important to make the notation "induced" on the specimen and requisition form since this may appear to be saliva to laboratory personnel.
    f. If only one or none of the first three smears are positive for AFB, then three more specimens should be collected. Smear reports require 1 business day following receipt of specimen.
    g. Should deliver within 24 hours but must be delivered within four (4) days. Store at 2° - 8° C.

2. Laryngeal Swabs:

    May be useful in children and in adults when no sputum can be raised. Submit in any commercially available swab transport system.

3. Gastric Lavage:

    If pulmonary secretions can not be collected as previously stated, patient should be hospitalized to collect gastric lavage. Perform lavage early in the morning before patient eats or gets out of bed or after a minimum 8 hour fast. After first collection, give 20-30 ml of water, and aspirate. The laboratory must be called when the procedure is to be done so preparations can be made for immediate processing (222-5587). Specimens of 5 – 10ml should be collected on three consecutive days. Deliver to the laboratory immediately, also include time collected on specimen container, collect in RI HEALTH Laboratories containers marked "For Gastric Specimens (AFB) Only." **Gastric specimens collected in other containers will not be accepted except by special arrangements.** Store at 2° - 8° C.

Other:

Bronchial washings, brushings and trans-tracheal aspirates produce primary specimens and cause patient to produce sputum naturally for several days. Continue to collect specimens for 1 to 2 days following bronchoscopy to enhance detection. Bronchial washings and trans-tracheal aspirates should be 5 ml or more. Should deliver within 24 hours, must be delivered within four (4) days. Store at 2° - 8° C.

C. EXTRA-PULMONARY DISEASE

Both chest x-ray and tuberculin skin test may be negative. Since Mycobacteria may infect any organ of the body, many types of specimens may be submitted including: aseptically collected body fluids, surgically excised tissues, pus, urine, and feces. These are divided into two groups:

- Aseptically collected specimens are usually free of microorganisms other than the immediate pathogen

- Specimens known to contain contaminating microflora or specimens not collected aseptically

1. Aseptically Collected Specimens:

A. Aseptically Collected Fluids

These include blood, bone marrow, pericardial fluid, synovial fluid, ascitic fluid, pleural fluid, spinal fluid and pus. All of the above are collected by the physician surgically or by aspiration. Should deliver within 24 hours but must be delivered within four (4) days. Store at 2° - 8°C.

Blood and bone marrow are collected in tubes containing SPS (yellow top) or Heparin (green top). Isolator tubes (Wampole) 10 ml adult or 1.5 pediatric are also acceptable. **EDTA (purple top) is unacceptable.**

Spinal fluid (CSF) is submitted in a tight stoppered sterile tube, optimally in 5-10 ml amounts (2ml minimum). Synovial fluid is submitted in the same manner as CSF and in similar quantities.

Pericardial, pleural, and peritoneal fluids are submitted in the sterile plastic 50ml centrifuge tubes supplied by RI HEALTH Laboratories. Sample volumes of 5 - 10 ml are preferred.

B. Aseptically Collected Tissues

Place in sterile container such as the 50-ml screw cap centrifuge tube provided with enough sterile saline to cover specimen. **Fixatives or preservatives are unacceptable.**

2. Specimens Expected to be Contaminated:

Most specimens received in the Mycobacteriology laboratory fall into this group and are handled in the same way as contaminated pulmonary specimens such as sputum, bronchial-washings, etc.

A. Urine

1. External genitalia should be washed prior to collection to minimize contamination
2. Three consecutive day first morning midstream voided urine, 20-50 ml. Should be collected in 50ml sterile screw cap centrifuge tube provided. **Twenty-four-hour pooled specimens are unacceptable.**
3. Store refrigerated at 2°-8°C. Should deliver within 24 hours but must be within four (4) days.

B. Feces

Feces can be shipped in the 50ml sterile centrifuge tube provided in the RI HEALTH Laboratories kit. A 5ml quantity is sufficient for testing. Should deliver within 24 hours but must be delivered within four (4) days.
Store at 2° - 8° C.

II. LABORATORY TESTING

A. Microscopy

1. All specimens receive an AFB smear within 1 business day following receipt of specimen.
2. Routine smears are stained with Auramine and read by fluorescence microscopy. Questionable smears may be re-stained by Kinyoun stain and re-read by standard light microscopy.
3. Positive AFB smears are telephoned and faxed to the submitter. A report is issued for both positive and negative smears.

B. Culture

1. All specimens are cultured in BACTEC bottles, on Lowenstein - Jensen slants, and 7H-11 agar plates.

    a. Readings are performed on BACTEC bottles daily; agar plates and slants are examined weekly.
    b. BACTEC bottles or solid media showing positive growth are tested by DNA probe for M.tuberculosis complex, M.avium complex, and/or others. In most cases, M.tuberculosis complex and M.avium complex can be identified in one to three days using this methodology.
    c. Some Mycobacteria require biochemical testing for identification and thus may take longer to identify. Unusual organisms or those yielding equivocal results will be referred to the Centers for Disease Control and Prevention (CDC).
    d. Submitters are called and reports issued when:
        1. Mycobacterium is first found in smear and/or culture.
        2. The organism is identified.

C. Drug Susceptibility Testing

1. Susceptibility tests are **not** routinely performed on M.avium complex since this organism is almost universally resistant to all the drugs tested. In vitro results have not been shown to correlate with in vivo results. Arrangements may be made to have these tested at the National Jewish Medical and Research Center.
2. M.fortuitum and M.chelonae will be forwarded to CDC for appropriate drug susceptibility testing.
3. M.tuberculosis complex and other pathogenic Mycobacteria are tested by either agar growth inhibition or BACTEC. Drugs tested are as follows:

    a. First Line Drugs

DRUG (mcg/ml)*	Agar Proportion Method	BACTEC
STREPTOMYCIN	2.0	2.0
STREPTOMYCIN	10.0	6.0
ISONIAZID	0.2	0.1
ISONIAZID	1.0*	0.4*
ETHAMBUTOL	5.0	2.5
ETHAMBUTOL	10.0	7.5
RIFAMPIN	1.0	2.0
RIFAMPIN	5.0*	----
PYRAZINAMIDE	25.0	100

    *Routinely only the critical (lower) concentration is used.

b. Second Line Drugs—used when organisms show resistance too more than one First Line Drug or by special arrangements.

KANAMYCIN 6.0
ETHIONAMIDE 5.0
CAPREOMYCIN 10.0
CYCLOSERINE 30.0
PAS 2.0
PAS 10.0
AMIKACIN 30.0
CIPROFLOXACIN 5.0
OFLOXACIN 5.0

4. Susceptibility testing may require one to four weeks following identification.

D. Reference Cultures

1. The Mycobacteriology Laboratory accepts reference cultures for identification and drug susceptibility testing.
2. Organisms should be submitted within 24 hours following isolation in the hospital to provide the fastest possible turn around time. **A minimal volume of 4ml of liquid is required for testing. A hard copy of the primary smear report must be submitted with the specimen.**

   a. Depending on the amount of growth submitted, age of culture, and whether further sub-culturing is required, expect 48 hours to four weeks for an identification and one to four weeks for drug susceptibility testing following receipt at RI HEALTH Laboratories.

E. Laboratory Reports

1. Positive smears, cultures, and identifications are telephoned and auto faxed to submitters.
2. Written reports are issued for:

   a. Positive and negative AFB smear results
   b. When Mycobacteria are first found in culture.
   c. When an identification is completed.
   d. When drug susceptibility testing is completed.
   e. When Mycobacteria are not found after eight weeks incubation.

SPECIMEN REJECTION

1. A specimen is unlabeled or mislabeled.
2. A specimen is more than 4 days old.
3. A Gastric specimen is not received in a state container with preservative unless prior arrangements made.
4. The name on specimen and the requisition **do not** match.
5. A specimen received without patients requisition form or, if the requisition form is not complete or blank.
6. The specimen is leaking.
7. The specimen is in a non-sterile container.

References:

Kent, PT, Kubica GP. Public Health Mycobacteriology 1985. A Guide for the Level III Laboratory, Centers for Disease Control, Atlanta, PHS, HHS.

Manual of Clinical Microbiology, 5th ed. American Society for Microbiology. Washington, D.C.

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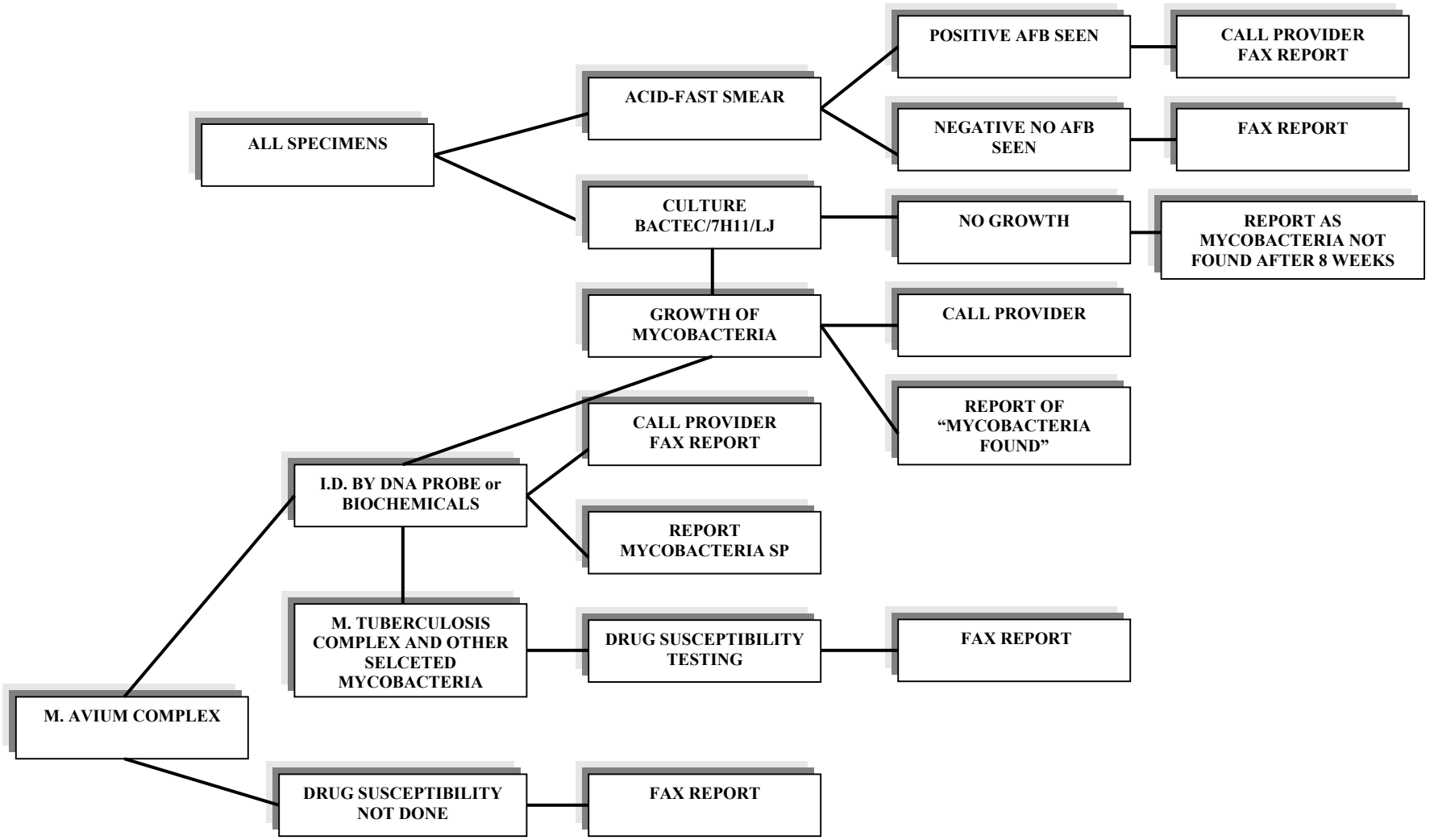

* * * PLEASE READ * * *

IMPORTANT INFORMATION BELOW

This is your revised guide to the services of the RI HEALTH Laboratories Mycobacteriology laboratory.

As you or your staff uses the Guide during the next 6 months, please note the topics, which are confusing or incomplete, and list items that you think should be added. Your comments will be used in preparing revisions and additions to the Guide.

Please send your suggestions to: **Melissa Martin, RI HEALTH Laboratories, 50 Orms Street, Providence, RI 02904-2284**

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[ ] I think the following suggestions would make the Guide more useful:

Page No.(s)	**Topic/Test**	**Comments**

[ ] I could not find the information I need about the following:

[ ] Please send an additional copy of this Guide to:

Name & Address _____

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